ATS LABS

PROTOCOL

Standard Test Method for the Evaluation of Laundry Sanitizers

Test Organism:

Pseudomonas aeruginosa (ATCC 15442)

PROTOCOL NUMBER

KIK02012213.LSAN.1

PREPARED FOR

KIK Custom Products
909 Magnolia Avenue
Auburndale, FL 33823

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

Joshua Luedtke, M.S.
Microbiologist

DATE

January 22, 2013

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

Standard Test Method for the Evaluation of Laundry Sanitizers

SPONSOR: KIK Custom Products
909 Magnolia Avenue
Auburndale, FL 33823

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this study is to evaluate the effectiveness of the Sponsor's test substance on reducing the microorganism population on fabric in a simulated laundry operation.

TEST SUBSTANCE CHARACTERIZATION
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is January 28, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of February 25, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs nor any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the regulatory agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
Regulatory agencies require that antimicrobial products which claim to provide sanitizing activity for fabrics and/or laundry water support this claim by providing appropriate scientific data demonstrating the efficacy of the test substance against the claimed organism. This is accomplished in the laboratory by treating the target organism with the test substance under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. For sanitization products intended for high efficiency, commercial or household use on fabrics and/or laundry water, a fabric carrier method is used in the generation of the supporting data. The test method to be used in this study will follow the simulated-use procedure employed by Petrocci and Clarke for the evaluation of antimicrobial laundry additives.

TEST PRINCIPLE

Fabric carriers are inoculated with a suspension of the test organism and dried. The inoculated fabric carriers are exposed to the test substance in a simulated laundry operation. Following exposure, the test carriers and wash water are assayed for survivors. Appropriate culture purity, sterility, carrier population, initial suspension, neutralization confirmation controls are performed. The current revision of SOP CGT-4045 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Pseudomonas aeruginosa	15542	Nutrient Agar A Slant and Nutrient Agar B	35-37°C, aerobic

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Preparation of Test Organism

The test organism will be transferred daily on Nutrient Agar A slants. The test organism will be transferred at least three but less than 16 transfers. The slants will be incubated for 24 ± 2 hours at 35-37°C. On the day prior to test, wash the growth from the slant using a 5.0 mL aliquot of Phosphate Buffer Dilution Water (PBDW). Aspirate the growth suspension and add it to a 99 mL aliquot of PBDW. Mix this solution. Add a 2 mL aliquot of the mixed suspension to each of four Nutrient Agar B culture bottles, minimally. Aspirate the excess liquid from the Nutrient Agar B bottles. Incubate the bottles for 18-24 hours at 35-37°C with the agar side down. On the day of test, harvest the test organism from the Nutrient Agar B bottles by adding a 2 mL aliquot of PBDW and approximately 15-20 sterile glass beads rocking the bottles back and forth. A spec value of approximately 1.7 at 620 nm should be targeted. Cultures may be further adjusted (concentrated or diluted), as needed, to ensure an appropriate carrier load.

An organic soil load may be added to the test culture per Sponsor's request.

Fabric Preparation

Prepare a scouring solution by adding approximately 1.5 grams Na_2CO_3 and approximately 1.5 grams of Triton X-100 to approximately 3 L of deionized water (or equivalent dilution). The test fabric will be plain cotton weave containing approximately 80 x 80 threads/inch. Add approximately 300 grams of test fabric to each 3 L volume of scouring solution, or equivalent. Allow the solution to boil for approximately 60 minutes. Remove the fabric and rinse by placing first in boiling water for a minimum of five minutes and then placing in cold water for a minimum of five minutes. During the rinsing procedure, mix the fabric in the water in order to help remove the wetting agent. Allow the fabric to air dry.

Carrier and Spindle Preparation

Cut the scoured and dried fabric into approximately 5 cm (2 inch) wide strips weighing 15±1 gram. Wrap each strip of fabric around a stainless steel spindle at least twelve, but fewer than thirteen times. Use a safety pin to secure the fabric. A pocket may be stapled between the sixth and seventh folds on the spindle to carry the swatch during testing procedures. Autoclave-sterilize fabric wrapped spindles, allow to cool and hold at room temperature.

Cut fabric swatch carriers of approximately 1 inch x 1.5 inch from scoured fabric. Place fabric carriers in a sterile vessel, autoclave sterilize, and allow to cool. Hold at room temperature until used in the test. Prior to sterilization, a plastic tag may be secured to each carrier to aid in removal from the spindle.

NOTE: Alternatively, Sponsor prepared spindles and fabric carriers may be utilized.

Preparation of Test Substance

The test substance is prepared according to the directions for intended use of the test substance. The test substance shall be used within three hours of preparation if additional preparation is required by ATS Labs. Fill the Nalgene or Mason jars with the prepared test substance according to the Sponsor specified test substance to fabric ratio (w/w) indicated on the Study Information Page. The test substance may be equilibrated to the exposure temperature prior to testing.

Desired Test Substance Use Pattern	Recommended Test Substance to Fabric Ratio (w/w)
Household Application	10:1 (for example 150 g ± 0.1 g of test substance per jar)
Commercial Application	5:1 (for example 75 g ± 0.1 g of test substance per jar)
High Efficiency Application	<5:1 (for example 60 g ± 0.1 g for 4:1 of test substance per jar)

Contamination of Carriers
Inoculate triplicate fabric swatch carriers, per test condition, with 30 µL aliquot of prepared test organism. Dry the carriers in a 35-37°C incubator for 10-15 minutes. Remove the fabric carriers from the incubator and place up to three carriers (per spindle) individually into the spindle pockets between the sixth and seventh folds of the spindles.

Exposure Conditions

For Household or Commercial Applications: Place the spindles containing the fabric carriers into the jars containing the test substance at the Sponsor requested use-dilution. Place the jars into a device to simulate tumble-wash at 45-60 RPM for the desired exposure time and temperature.

For High Efficiency Applications: Prior to exposure, aseptically remove the spindle wire from the fabric spindle. Place the fabric spindle containing the carriers into the jars containing the Sponsor specified volume of test substance. Place the jars into a device to simulate a tumble-wash at 45-60 RPM for the desired exposure time and temperature.

Test System Recovery
Following completion of the simulated wash, prior to the end of the exposure time, remove the spindle from the jar. Fully wring the liquid from the spindle to ensure elution of survivors into the wash water. Aseptically remove the fabric carriers and place individually into jars containing 10 mL of the neutralizer at the end of the exposure time. Transfer 1.0 mL of the "wash" water to a vessel containing 9 mL of the neutralizer. Vortex mixed the subcultures for a minimum of 10 seconds. Prepare ten-fold serial dilutions and spread plate 1.0 mL of the 10^0 to 10^{-4} dilutions in duplicate. If swarming is a concern, spread plate 1.0 mL aliquots of 10^0 and 0.1 mL aliquots of the 10^0 through 10^{-3} dilutions in duplicate.

Incubation and Observation
Incubate all plates at 35-37°C for 48 ± 4 hours prior to reading. Following incubation, the subcultures may be stored at 2-8°C for up to three days prior to reading. Use standard plate count procedures to determine the average colony forming units per carrier, and per milliliter of wash water.

Representative test plates showing growth may be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. If possible, subcultures containing 30-300 colonies will be used for calculations.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Load Sterility Control
If applicable, the serum used for soil load will be cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier will be added to the neutralizer. The neutralizer containing the carrier will be mixed and 1.0 mL will be spread plated. The plate will be incubated as in the test and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A 1.0 mL sample of uninoculated neutralizer will be plated, incubated as in the test and visually examined. The acceptance criterion for this study control is lack of growth.

Carrier Population Control
Three contaminated carriers will be treated using test substance diluent in place of the test substance. (Butterfield's buffer or PBDW may be used if the test substance is ready to use.) Expose the carriers for the highest exposure time and/or temperature, as in the test. Following exposure, fully wring the liquid from the spindle to ensure elution of survivors into the wash water and neutralize the carriers and wash water as in the test. Vortex mix the subcultures for a minimum of 10 seconds. Prepare ten-fold serial dilutions and spread plate 1.0 mL of the 10^{-1} to 10^{-5} dilutions in duplicate. If swarming is a concern, spread plate 0.1 mL aliquots of the 10^{0} through 10^{-4} dilutions in duplicate. The acceptance criterion for this study control is a minimum geometric mean value of 1×10^{4} CFU/Carrier and a minimum value of 1×10^{4} CFU/mL in the wash water.

Initial Suspension Control
Dilute the prepared inoculum serially in ten-fold increments and plate aliquots from selected dilutions on agar plates in duplicate. Incubate plated samples as in the test before reading. Use standard plate counting procedures. There is no acceptance criterion for this control.

Neutralization Confirmation Control
Sterile test carriers will be exposed to the test substance as in the test procedure. More than three sterile carriers may be exposed per spindle. Only the most concentrated test substance and/or shortest exposure time needs to be utilized in this control. The carriers will be neutralized as in the test procedure. A 1.0 mL aliquot of test organism will be added to the vessel to yield approximately 100 CFU per mL of neutralized material. Multiple dilutions of the test organism may be utilized. The vessels will be mixed and 1.0 mL aliquots will be plated in duplicate onto an appropriate agar and the plates will be incubated. Perform a numbers control using untreated neutralizer. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the numbers control.
If swarming is a concern, duplicate 0.1 mL aliquots will be plated. In this case, sufficient organism will be added to the vessels to target approximately 1000 CFU per mL of neutralized material.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM
ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

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STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The U.S EPA efficacy data requirements state that an effective laundry sanitizer reduces the bacteria on the fabric carrier and the wash water by at least 99.9% over the corresponding population control.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES
If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION
It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product *Performance Test Guidelines, OCSPP 810.2000: General Considerations for Public Health Uses of* Antimicrobial Agents, March 12, 2012.
2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles- Efficacy Data Recommendations, May 30, 2012.
3. Petrocci, A.N. and Clarke, P., "Proposed Test Method for Antimicrobial Laundry Additives:, Journal of the AOAC, Vol. 52 No. 4, 1969, pp 836-842.
4. "Bacteriostatic Activity of Laundry Additive Disinfectants", AOAC Official Methods of Analysis, 18th edition, Section 6.3.07, 2005, Chapter 6.
5. "Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants", ASTM Standards, 2009 Volume 11.05, Designation E2274-09
6. "Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants for Use In High Efficiency Washing Operations", ASTM Standards, 2009 Volume 11.05, Designation E2406 – 09.

DATA ANALYSIS

Calculations

Initial Suspension Control CFU/mL=$\frac{\text{(average number of colonies/plate @ dilution used) (dilution factor)}}{\text{(volume plated)}}$

Number of Organisms Surviving: Carrier

CFU/carrier = $\frac{\text{(average number of colonies/plate @ dilution used) (dilution factor) (volume of neutralized solution)}}{\text{(number of carriers tested)}}$

The carrier population will be calculated and reported using data from the most appropriate dilution(s).

Number of Organisms Surviving: Wash Water

CFU/mL = $\frac{\text{(average number of colonies/plate @ dilution used) (dilution factor) (volume of neutralized solution)}}{\text{(volume plated)}}$

The wash water population will be calculated and reported using data from the most appropriate dilution(s).

Geometric Mean of Number of Organisms Surviving: Carrier

Geometric Mean = Antilog of $\frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \ldots\ldots\text{Log}_{10}X_N}{N}$

Where: X equals CFU/carrier
N equals number of test or control carriers

Percent Reduction

% reduction = [(a - b) / a] x 100

where:
a = geometric mean of the number of organisms surviving on the inoculated control carriers or CFU/mL in the control wash water.
b = geometric mean of the number of organisms surviving on the test carriers or CFU/mL in the test wash water.

Statistical Analysis:
None used.

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
PURE BRIGHT PERMICIDAL ULTRA BLEACH* - 13022095SM , 13025071 9M, 12349220 6M*

Expiration Date: AUG. 1, 2013 *EPA Reg. No. 70271-13 *60 DAY SAMPLE PRODUCED DEC. 15, 2012 60 DAYS OLD ON FEB. 13, 2013

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☑ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☐ Other

Test Substance Active Concentration (upon submission to ATS Labs): 6%

Neutralization/Subculture Broth:
- ☐ _____
- ☒ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions
- ☒ Room Temperature
- ☐ 2-8°C
- ☐ Other _____

Hazards
- ☐ None known: Use Standard Precautions
- ☒ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

Product Preparation **SEE PROTOCOL MODIFICATION
- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:
  1 and 1/4 cup per 16 gallons (example: 1 oz/gallon) defined as 1 1/4 CUPS (**amount of test substance) + 16 GALLONS (amount of diluent)
  - ☐ Deionized Water (Filter or Autoclave Sterilized)
  - ☒ Tap Water (Filter or Autoclave Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____ PPM
  - ☐ Other _____

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Substance-to-Fabric Ratio (w/w): 10:1

Intended Application: ☒ Household ☐ Commercial ☐ High Efficiency

Test Organism(s): ☑ *Pseudomonas aeruginosa* (ATCC 15442)

Number of Carriers: 3 per batch

Exposure Time: 10 Minutes Exposure Temperature: 20 °C (±2°C)

Organic Soil Load:
- ☒ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☐ No Organic Soil Load Required
- ☐ Other _____

* part of test substance name per email on 1-31-13 JDR 2-14-13

TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in ~~one or~~ more previous studies at ATS Labs.
- ☐ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs:_____ Sent via *overnight* delivery? ☐ Yes ☐ No
- ☒ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs: FEB. 5, 2013
- ☐ Sender (if other than Sponsor):_____

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

- ☐ Approved without modification
- ☑ Approved with modification

Prior to testing, perform the following per product preparation, per day: Titrate test substance per ATS Labs SOP CGT-0090 to determine starting sodium hypochlorite concentration. Dilute test substance to 56,500-57,000 ppm with sterile tap water and titrate to confirm. Dilute test substance per page 9 of protocol for use in testing.

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Mr. Justin Lowe TITLE: Regional QA Manager

SIGNATURE: Justin Lowe DATE: 1/29/2013

PHONE: (863) 551 - 3006 FAX:_____ EMAIL: jlowe@kikcorp.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

CRISTINA GRIFFIN - CGRIFFIN@DELTA-AC.COM

ATS LABS:

NAME: Jill Ruhme
Study Director

SIGNATURE: Jill Ruhme DATE: 2-14-13
Study Director